INTRODUCTION

C-reactive protein (CRP) is elevated in patients with stable coronary artery disease demonstrated by angiography, and its serum values increase notably in acute coronary events like unstable angina and acute myocardial infarction. All authors agree that an abrupt increase in inflammatory status leads to an elevation of the markers cited which, in turn, induces the activation of coagulation, thus generating thrombin and increasing intra-arterial thrombosis. Nevertheless, there is the possibility that circulating thrombin, in turn, produces an elevation in inflammatory markers, closing a feedback loop that would explain the high elevation of CRP and cytokines in patients with arterial thrombosis. In this study we evaluated blockade of thrombin generation by means of a glycoprotein (GP) IIb/IIIa receptor (eptifibatide) to determine if it reduces CRP elevation after coronary angioplasty.

PATIENTS AND METHOD

This study is designed prospectively with all the inclusion and exclusion criteria determined previously.

We measured C-reactive protein concentrations in 31 patients with interventional procedures after blocking thrombosis in 17 of them by the administration of a 12 hour long infusion of eptifibatide in order to evaluate the role of arterial thrombosis. There were no differences in C reactive protein concentration between the treated and control group pre-angioplasty (0.32 ± 0.4 vs 0.56 ± 0.57 mg/dl; p = NS), nor post-angioplasty (0.35 ± 0.42 vs 0.53 ± 0.5 mg/dl; p = NS). The eptifibatide group maintained basal C reactive protein concentrations 6 hours after the procedure, while the control group had a significant increase (0.43 ± 0.5 vs 1.02 ± 0.89 mg/dl; p < 0.05). There was a decrease in C reactive protein 24 hours after angioplasty in eptifibatide group (0.24 ± 0.27 vs 1.34 ± 0.89 mg/dl; p < 0.001), but it increased again 48 hours after the procedure although to a lesser extent than in the control group (0.57 ± 0.55 vs 2.18 ± 2.1 mg/dl; p < 0.05). Eptifibatide, a synthetic peptide which is a selective blocker of the platelet GP IIb/IIIa receptor significantly reduced C-reactive protein concentration after angioplasty.
Patients and study design

The study included 31 consecutive patients in which coronary angioplasty was performed in our hemodynamics laboratory. Patients with diseases that could present high CRP values were excluded: inflammatory, infectious, neoplastic, endocrine, and metabolic diseases, recent surgery or acute myocardial infarction (<3 months), patients with angioplasty on a venous graft and angioplasty in acute myocardial infarction.

The patients were included consecutively in both groups. First, 17 patients under treatment were included and then the 14 patients who constituted the control group were included. All patients followed the usual angioplasty protocol of our center. The group of 17 treated patients received a bolus of 180 µg/kg i.v., and a perfusion of eptifibatide 2 µg/kg/min i.v. at the end of angioplasty, immediately after extracting the post-procedure blood sample. The eptifibatide perfusion was maintained for 12 h and was not continued later in order to determine if there was a new increase in CRP as a result of renewed thrombin generation. The control group of 14 patients received conventional treatment.

Laboratory analysis and sample collection

Blood samples were extracted before and after angioplasty, and 6 h, 24 h, and 48 h after the procedure. CRP samples were obtained in biochemistry tubes without coagulant and centrifuged at 2000 (10 min. They were analyzed in our center by nephelometry. The limit of normality was 0.3 mg/dl.14

Statistical analysis

The comparison between groups was made using the non-parametric Mann-Whitney test and ANOVA with the Friedman test for comparison of means between groups. The significance level was P<.05.

RESULTS

Both groups were similar in age (control 65.1±10.2 years; eptifibatide 62.7±8.8 years; P=NS), body surface area (1.8±0.18 m² versus 1.95±0.17 m²; P=NS), history of arterial hypertension (42.8% versus 47%; P=NS), smoking habit (21.4% versus 47%; P=NS), and hypercholesterolemia (21.4% versus 29.4%; P=NS). Diabetic patients were excluded. There were no differences in the clinical presentation: 9 of 14 patients in the group control (64.3%) and 9 of 17 patients in the treated group (52.9%) had unstable angina (P=NS). The angiographic characteristics of the lesion also were similar in the two groups. A B2 or C lesion was present in 64.3% of the control group versus 62.5% of the treated patients (P=NS). Lesions with irregular edges were present in 71.4% of controls and 75% of treated patients (P=NS). There were no differences in the severity or final diameter of the lesion measured by quantitative angiography (pre-procedure stenosis: control 82.8±14.1% versus eptifibatide 83.1±11.5%; P=NS) (post-procedure stenosis: control –2.53±8.4% versus eptifibatide –1.7±4.5%; P=NS). The maximum dilation pressure also was similar (control 18.1±4.1 atm versus eptifibatide 19.5±3.4 atm; P=NS). No patient presented high CK/MB levels after the procedure. CRP values after angioplasty in both groups are shown in Table 1.

DISCUSSION

C-reactive protein and coronary disease

The increase in C-reactive protein values has been related with persistent subclinical instability of the atheroma plaque. CRP elevation in healthy subjects and patients with stable angina is associated with a higher incidence of cardiovascular events.1-10 Patients with unstable angina and elevated CRP present a significant increase in the risk of recurrent ischemia, infarction, and death.5-8 Finally, in coronary angioplasty the atheroma plaque fractures, turning a stable lesion into an unstable one, and C-reactive protein increases, peaking at 24-48 h and normalizing at 72 h.15

Proposed mechanisms of C-reactive protein elevation in coronary artery disease

The current explanation describes CRP elevation in coronary patients or after angioplasty or induction of an «acute inflammatory episode».1-10 Cardiovascular

### TABLE 1. Statistical analysis of C-reactive protein values in the two groups

<table>
<thead>
<tr>
<th></th>
<th>Pre-PTCA</th>
<th>Post-PTCA</th>
<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
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<tr>
<td><strong>Eptifibatide</strong></td>
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<tr>
<td>0.32±0.4 (1.5-0.03)</td>
<td>0.35±0.42 (1.6-0.03)</td>
<td>0.43±0.5 (2.1-0.05)</td>
<td>0.24±0.27 (0.8-0)</td>
<td>0.57±0.55 (1.61-0)</td>
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<tr>
<td><strong>Control</strong></td>
<td>0.56±0.57 (2.12-0.1)</td>
<td>0.53±0.5 (2.0-0.1)</td>
<td>1.02±0.89 (3.1-0.41)</td>
<td>1.34±0.89 (3.76-0.67)</td>
<td>2.18±2.1 (4.31-0.57)</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>NS</td>
<td>.05</td>
<td>.001</td>
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PTCA indicates coronary angioplasty. Values are expressed as mean±standard deviation and, in parenthesis, the range of values.
risk factors induce the secretion of macrophage colony-stimulating factor (MCSF) by the endothelium. The MCSF stimulates the production of more MCSF and IL-1 by the same endothelium and the macrophages of the arterial wall, which favors monocyte adhesion to the endothelium, which, in turn, release cytokines.\textsuperscript{10,12} The macrophages/cytokine-activated monocytes produce large amounts of IL-6 that, when released into the bloodstream, induce the production of CRP by hepatocytes, endothelial cells and macrophages.\textsuperscript{10,12} This could be the mechanism underlying the high MCSF, IL-6, and CRP values in patients with atherosclerosis, but it does not fully explain why these markers are higher in acute coronary syndromes than in stable patients.

We propose that the relation between inflammation and thrombosis is reciprocal. The elevation of CRP, IL-1, and IL-6 has procoagulant effects in general and on the atherosclerotic lesion.\textsuperscript{10,11} CRP also induces the formation of tissue factor by monocytes.\textsuperscript{12} Coagulation is activated by both routes, ultimately leading to thrombin formation in large amounts, and to a very active intra-arterial thrombogenic situation. Thrombin stimulates the release of IL-1 by macrophages,\textsuperscript{13} which in turn induces the production of large amounts of CRP by the macrophages themselves, endothelial cells, and hepatocytes. This would activate the coagulation system again, forming a closed feedback loop between arterial inflammation and thrombosis.

Recent findings support a relation between the activation of coagulation and CRP elevation. Ikonomidou et al studied 40 patients with demonstrated stable coronary artery disease and high CRP (>0.3 mg/dl) and found a mean reduction in CRP levels from 1.25 to 0.23 mg/dl after a month of treatment with 300 mg/day of aspirin.\textsuperscript{10} In a retrospective analysis, abciximab reduced CRP values after angioplasty in a subgroup of patients with unstable angina in the EPIC study.\textsuperscript{16} Finally, in patients with severe sepsis who present an exacerbated inflammatory situation and elevated CRP, a decrease in acute-phase reagents is observed (including CRP) when a thrombin antagonist like antithrombin III is administered.\textsuperscript{17}

**Study design and analysis of results**

The sequence of events in this study were designed to fit as closely as possible the sequence of events that occur in acute coronary syndrome. In first place, we have baseline CRP values. Later, we determined the exact moment of induction of arterial thrombosis by plaque fracture during coronary angioplasty. Since CRP begins to rise 6 h after the stimulus and has a mean life of 19 h, we took blood samples in the previous time intervals.\textsuperscript{15} Next, we administered eptifibatide to the treated group after producing the thrombogenic stimulus by angioplasty, for the purpose of observing if the drug was capable of blocking CRP elevation through the stimulus induced. Finally, we discontinued eptifibatide perfusion at 12 h to determine if CRP again rises after the mean half-life of the drug (2 h to 4 h) as a result of new thrombin generation.

Both groups were similar in age, body surface, cardiovascular risk factors, clinical presentation, and angiographic characteristics of the lesion. No enzyme elevation was appreciated in any patient.

Baseline CRP (pre and post-angioplasty) was similar in both groups. A significant difference was observed at 6 h: in the group treated with eptifibatide the CRP ascent normally observed after angioplasty was blocked. At 24 h, the difference was significant (Table 1). Forty-eight hours after angioplasty and 36 h after concluding the eptifibatide perfusion, CRP had again formed in the treated group.

Eptifibatide is a peptide that selectively blocks the GP IIb/IIIa receptors of the integrin family, which are found only in platelets and megakaryocytes. Eptifibatide blocks the KGD zone and anchor zone of the fibrinogen chain, and does not have well-known anti-inflammatory effects. It has a half-life of 2 h to 4 h and is eliminated by the kidney.\textsuperscript{15} The GP IIb/IIIa receptor is the common pathway of platelet aggregation, regardless of the mechanism of activation. Aggregation is accompanied by the release of substances that promote more aggregation and thrombin formation, with subsequent activation of the coagulation system. The established doses of eptifibatide are designed to block 80% of platelet receptors, obtaining a significant (but not complete) reduction of platelet aggregation and thrombin generation. In our study, CRP did not increase and it even decreased in some patients with eptifibatide perfusion. In contrast, it increased 36 h after discontinuing eptifibatide perfusion, indicating a relation between eptifibatide treatment and CRP values. This relation cannot be due to anything except the antithrombotic effect of the drug.

**Study limitations**

The number of patients included in the study was small, but the difference between mean values is evident, which is why the results must be considered valid. Nevertheless, more studies should be made to confirm these results by analyzing mediators of CRP production like IL-6 and other cytokines, in addition to a parameter to quantify thrombin production.

**CONCLUSIONS**

Eptifibatide blocks the increase in C-reactive protein values that is routinely observed after coronary angioplasty. Since this drug selectively and specifically blocks the GP IIb/IIIa receptor, and has no other
known effect, it must be concluded that the mechanism by which CRP increases after angioplasty is of a thrombotic type.

REFERENCES


Merino Otermin A, et al. Eptifibatide reduces C-reactive protein